THE BACTERIOLOGY OF APPENDICITIS AND ITS PRODUCTION BY INTRAVENOUS INJECTION OF STREPTOCOCCI AND COLON BACILLI*

EDWARD C. ROSENOW

(From the Memorial Institute for Infectious Diseases, Chicago, Illinois)

(WITH PLATES 12 TO 16)

The causes of appendicitis, exclusive of foreign bodies within the lumen of the appendix or other mechanical factors, are still obscure. The excellent, recent studies of Heyde[1] and others on the fluid contents of the appendix and peritoneum show the important rôle anaerobes probably play, at least as secondary invaders. The histological studies of Aschoff[2] emphasize the importance of streptococci and demonstrate that infection may pass from the lumen directly into the wall, especially if fecal stones are present. Adrian,[3] Kretz,[4] and Cannon[5] have observed cases of appendicitis following angina, an observation since repeated many times, and they suggest that appendicitis is a blood infection. Poynton and Payne[6] have reported appendicitis associated with arthritis in two of thirteen rabbits injected intravenously with the so-called Str. rheumaticus and in one of six rabbits injected with a streptococcus isolated from the inflamed appendix in a case of rheumatism. They were unable, however, to produce appendicitis with strains from the throat in the latter case. Ghon and Namba[7] have shown that spontaneous lesions of the appendix of rabbits occur and that appendicitis rarely develops in pyemia, and they suggest, with Aschoff, that if appendicitis begins commonly as an embolic infection then appendicitis must be due to a specific streptococcus or other organism having an elective affinity for the appendix. I[8] have observed that the streptococci from rheumatism, especially after animal passage, and also streptococci from other sources, when they have acquired a certain grade of virulence, occasionally produce appendicitis in rabbits on intravenous injection.

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1. Beitr. z. klin. Chir., 1911, 76, p. 1.
2. Ergebn. d. inn. Med. u. Kinderh., 1912, 9, p. 1.
3. Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1901, 7, p. 407.
4. Ztschr. f. Heilk., 1907, 38, p. 2960.
5. Deutsch. Ztschr. f. Chir., 1908, 95, p. 21.
6. Researches on Rheumatism, New York, 1914.
7. Beitr. z. path. Anat. u. z. allg. Path., 1912, 52, p. 130.
8. Jour. Infect. Dis., 1914, 14, p. 61.

As yet, no one has demonstrated that bacteria from a possible focus of infection, as the tonsil, in appendicitis will often localize in the appendices of animals when injected intravenously. It occurred to me that a careful, differential, bacteriological study of the fluids and tissues in and about the appendix and of the tonsils and other foci, with intravenous injections into animals of the isolated strains, might throw light on the question of the development of appendicitis.

TECHNIC

As soon as removed, the appendix was covered with sterile gauze. The cultures were usually made as soon as possible, but, in some instances, the appendix was put in the refrigerator overnight and cultures were made the following morning. Small pieces of the peritoneal coat and mesentery showing inflammation were removed in a sterile manner either with small scissors or scalpel. The appendix was then opened, the contents drawn into a sterile pipette, and a small portion of the wall removed after the mucous membrane had been thoroughly washed in sterile, running water. The tissues were then washed in sterile salt solution and emulsified with 5 c.c. of dextrose broth in a sterile mortar.

Inoculations of similar amounts of the emulsion and pus, or other material, were made on blood agar plates, ascites dextrose agar plates or in tall tubes, on anaerobic blood agar slants, and into tall columns (9-11 cm.) of ascites dextrose broth in tubes or bottles with or without sterile tissue. The fluid from the edematous appendical wall was sometimes inoculated into sterile, undiluted serum. It is important to use tall columns of broth so that the growing bacteria have a wide range of oxygen pressure, especially if selective localization is to be obtained.

The cultures were incubated at 35-37 C. from eighteen to twenty-four hours. They were then centrifugated, the broth poured off, and suspensions made in salt solution in such a way that 1 c.c. of suspension was equivalent to the growth from 15 c.c. of broth. The cultures from the tonsils were made in a similar way. In the tables, unless otherwise indicated, the number of cubic centimeters represents the growth in ascites dextrose broth. Most of the injections were made with mixtures of all the organisms which developed in the primary cultures in ascites dextrose broth and the organisms isolated from the lesions in the rabbits.

The injections were rapidly made in the marginal ear veins of rabbits with an ordinary hypodermic needle. No attention was paid to

the feeding time or character of the food, altho the injections were usually made late in the afternoon some hours after the animals were fed for the day. Control cultures of the suspensions were made just before the injections. This is important, because negative results were found often to be due to the fact that only dead bacteria were injected.

The animals were examined as soon after death as possible. This is also important because after death the appendix is quickly invaded by bacteria from the lumen. A painstaking search for lesions was made. In the case of joints, periarticular hemorrhages and turbidity of the articular fluid were regarded as due to arthritis. In the tables, the number of plus signs (+) roughly indicates the amount of lesion in the different organs. The early lesions were usually localized hemorrhages; the later, often localized edema and necrosis.

The cultures from the appendix and tissues in the animals were made either by emulsifying the tissues in a mortar or by drawing them into a pipette.

Tissues were fixed in 10 percent formalin or Zenker's fluid, stained with hematoxylin and eosin by the Gram-Weigert method. When the colon bacillus was not decolorized completely it could be easily recognized.

REVIEW OF CASES, CULTURE RESULTS, AND ANIMAL EXPERIMENTS

CASE 128.—Recurrent appendicitis and tonsillitis in a boy 12 years old. Present attack of appendicitis not definitely connected with an acute tonsillitis. Appendix, tonsils, and adenoids removed August 21 by Dr. Balfour. The appendix is hyperemic, free from constricting bands and fecal concretions, and contains a small amount of mucopurulent material; a lymph gland the size of a large pea is found at its beginning; the mucous membrane shows marked hyperemia and numerous punctate hemorrhages, especially near the tip, but it is not ulcerated.

Cultures from the lumen of the appendix yield colon bacilli; from the wall, colon bacilli and feebly green-producing streptococci; from the gland, feebly green-producing streptococci; and from the tonsils, feebly hemolysing and green-producing streptococci. The streptococci from the wall and gland are identical and resemble very closely the green-producing streptococcus from the tonsils.

Sections of the appendix show moderate hemorrhagic and leukocytic infiltration, which are most marked in the mucosa, submucosa, and lymph follicles. The mucous membrane shows a few streptococci and bacilli; the deeper layers show a few diplococci.

Intravenous injection of streptococci from the tonsil (Rabbits 686, 689, 692, 693, 702, and 703), mixed cultures of the colon bacillus and streptococcus from the wall of the appendix (Rabbit 705), and pure cultures of the latter (Rabbits 709, 710, 713, and 714) were followed, as shown in Table 3, by lesions in the appendix in all but two of the animals injected (Rabbits 692 and 702), while pure cultures of the colon bacillus failed to produce lesions in the appendix (Rabbits 687 and 704). The streptococcus from the appendix, when passed through ani-

mals, lost its affinity for the appendix and acquired an affinity for the mucous membrane of the stomach and duodenum (Rabbits 719, 720, 724, 728, Dog 131, and Pig 1234).

Rabbit 686.—Injected intravenously on August 23 with the growth from 75 c.c. of ascites dextrose broth of the original culture from the emulsified tonsil.

August 24, seems quite well; chloroformed and examined at once. Multiple hemorrhages of mucous membrane of appendix particularly over base. No hemorrhages in small or large intestine. A few small punctate hemorrhages of mucous membrane of cardiac end of stomach. No other gross lesions. August 25, bile and joint fluid sterile. Pure culture green-producing streptococcus from blood. Sections of the appendix show small hemorrhages just beneath the mucosa.

Rabbit 705.—Injected intravenously on August 26 with the mixed growth from 15 c.c. ascites dextrose tissue broth of streptococcus and colon bacillus from the wall of the appendix.

August 27, found dead. Approximately fifty small punctate hemorrhages in the mucous membrane of the appendix. A few hemorrhages in the right ventricle. No other gross lesions except marked, cloudy swelling of heart and kidney. August 29, large number of colon bacilli and streptococci from the blood and wall of appendix. Sections of the appendix show moderate number of small hemorrhages just beneath the mucosa and in the lymph follicles.

Rabbit 709.—Injected intravenously on August 28 with the growth from 60 c.c. of ascites dextrose broth of the streptococcus from the wall of the appendix.

August 31, seems quite well; chloroformed and examined at once. Large number of small, fading, punctate hemorrhages in the mucous membrane of the appendix surrounded by hyperemia. Joint fluid turbid; no other gross lesions. September 2, joint fluid sterile. A pure culture of distinctly green-producing streptococcus from blood.

Rabbit 719.—Injected intravenously on September 1 with the growth from 45 c.c. of ascites dextrose broth of the streptococcus from the appendix after one animal passage.

September 3, seems ill; chloroformed and examined at once. Four punctate hemorrhages in the duodenum in an area 3 mm. in diameter. The intervening mucous membrane seems swollen and opaque. One ulcer, 3x4 mm., in the stomach 2 cm. from the pyloric ring. The ulceration extends through the mucous membrane, the margins are abrupt, the base hemorrhagic. Similar ulcer, 2x3 mm., in the lesser curvature. Joint fluid very turbid; no other gross lesions. September 4, blood and joint fluid, sterile; bile, large number streptococci; ulcer, forty colonies of streptococci, pure.

CASE 184.—Acute gangrenous appendix in a girl, 17 years old. Present attack began four days ago with acute pain in abdomen which was rather diffuse at first, but later became localized in the right lower quadrant and was associated with nausea and vomiting. She had a similar, but milder, attack one month before. There has been no history of sore throat, but since the last attack she has developed a cough and raises a moderate amount of sputum. On October 31, a gangrenous appendix embedded in a mass of edematous adhesions was removed by Dr. C. B. Davis. On October 31, appendix is much swollen, dark red, and nearly perforated near the base. The lumen contains a moderate amount of bloody pus, without foul odor, and is free from fecal concretions and foreign bodies. The mucous membrane is necrotic throughout.

On November 2, cultures from the lumen yield chiefly colon bacilli and a few streptococci. Blood agar plate cultures from the hemorrhagic peritoneal coat near the tip showed 45 colonies and from the peritoneal coat near the base, 3,600 colonies of a streptococcus, which produces a distinct green and a narrow zone of hemolysis peripheral to the green zone. A pure culture of the same streptococcus is found also in ascites dextrose broth and agar and in the anaerobic cultures. The wall of the appendix shows chiefly this streptococcus but also a moderate number of colonies of the colon bacillus. No fusiform bacilli or spirilla.

Sections from the appendix show marked hemorrhage and leukocytic infiltration throughout the wall, but principally in the submucosa, the lymph follicles, and subperitoneal coat. In many places, the connective tissue and muscle fibers are widely separated by large areas of hemorrhage in which are relatively few leukocytes. Gram stains show moderate number of gram-positive diplococci and short chains. These are found both inside and outside of the leukocytes. The streptococci are most numerous in areas where necrosis and leukocytic infiltration are pronunced. Sections stained by Greenwald's blood stain show fully one-third of the polynuclear leukocytes to be eosinophils. A few bacilli corresponding to colon bacilli are found in the mucous and submucous layers. No thrombosis of vessels can be made out.

On November 2, the tonsils are small, ragged, and a small amount of pus (1 c.c.) is expressed from a deep crypt in the upper pole of the right tonsil. The cultures, on November 3, in ascites dextrose broth of the pus from the tonsil show a large number of short-chained, non-clumping streptococci and what appear to be colon bacilli. The blood-agar plates show chiefly the streptococcus viridans, a few hemolytic streptococci, and colonies resembling colon bacilli.

The tonsil was again examined on November 6 and a similar quantity of pus expressed from the same crypt. November 7, the cultures in ascites dextrose broth, instead of producing a diffuse turbidity containing short chains of streptococci, produce a marked flocculent sediment composed of extremely long chains of streptococci, often in large clumps. The bacilli resembling the colon bacillus are absent. Blood agar plate made directly from the material from the tonsil shows chiefly the streptococcus viridans and a few hemolytic streptococci and staphylococci. A culture from the sputum shows a moderate number of colonies of streptococci, both hemolysing and green-producing, and colon bacilli.

A small amount of pus is again expressed from the crypt on November 24 in the right tonsil and cultures made. The patient is now well and is about to leave the hospital.

The results of the intravenous injection, as shown in Table 4, are as follows: The first culture from the edematous peritoneum (Rabbits 785, 851, 852, 853, 854, 855, 864, 865, and 866) containing a pure culture of streptococci shows a striking affinity for the appendix. The colon bacillus from the appendix showed no special predilection for the appendix (Rabbits 885 and 886). The streptococcus isolated from the appendix of rabbits, injected with the mixed cultures of streptococcus and colon bacillus isolated both from the tonsil and appendix in the patient, show similar, but less striking, lesions; hemorrhages of the stomach and duodenum were more common (Rabbits 862, 863, 868, 869, 870, 877, 878, 879, and 880). Rabbit 883 showed lesions in the appendix, stomach, and gall-bladder. The third animal passage of the streptococcus, originally isolated from the appendix, produced no lesions of the appendix, but showed

an affinity for the gall-bladder and joints instead (Rabbits 875 and 894). Pure cultures of the streptococcus from tonsil forty-eight hours after operation produced marked hemorrhages of the appendix only (Rabbit 890). Pure cultures of the colon bacillus produced only slight hemorrhages of the appendix, duodenum, and small intestines (Rabbits 885 and 886). The elective affinity for the appendix of the mixed cultures of the streptococcus and colon bacillus lasted for nine days (Rabbits 887 and 888), while the pure cultures of the streptococcus from the tonsil had nearly lost it (Rabbit 829), and the one from the appendix had entirely lost it (Rabbit 831).

As the streptococci in the test tube lost the affinity for the appendix, those in the crypt of the patient's tonsil also lost their affinity, because cultures of the pus expressed six and twenty-five days after the operation failed to show the slightest tendency to infect the appendix, but now produced neither lesions nor arthritis, endocarditis, or myocarditis (Rabbits 881 and 882). The recovery of the colon bacillus from the tonsil was unusual and, because the lesions in the appendix with mixtures of the streptococcus and colon bacillus were so striking, it was thought that possibly the streptococcus had acquired this peculiar property from growth in symbiosis with the colon bacillus. The streptococcus from the tonsil in the second culture, which showed no tendency to infect the appendix, was now grown in ascites dextrose broth with the colon bacillus. The mixture was then injected, but no lesions of the appendix developed (Rabbits 891 and 893).

Rabbit 847.—Injected intravenously on November 1 with the growth from 1.5 c.c. ascites dextrose tissue broth inoculated with the emulsion of the appendix and containing streptococcus and colon bacillus.

November 3, seems quite well; chloroformed. No gross lesions except a moderate number of punctate hemorrhages in the cortex of the kidney and three poorly circumscribed areas of infection associated with hyperemia and edema in the mucous membrane of the appendix. Each of these is situated in the more avascular portion of the appendix where the anastomosis of the blood vessels occurs.

November 5, cultures from the areas of infection in the appendix show a large number of streptococci and only a few colonies of colon bacilli, while the blood yields an almost pure culture of streptococcus, a few colonies of colon bacilli, and, in addition, the shake cultures in ascites dextrose agar yield the bacillus welchii.

Rabbit 857.—Injected intravenously on November 3 with the growth from 15 c.c. of ascites dextrose broth from the tonsil containing streptococcus and colon bacillus.

Found dead on November 4. The appendix is almost black with a large number of hemorrhages, chiefly in the submucosa and subperitoneal coat. Moderate number of smaller punctate hemorrhages at the ileocecal valve, the lymph follicles of the small intestines, a few in the mucous membrane of the duodenum, but none in the stomach, kidneys, adrenals, heart, brain, liver, spleen, eyes, or testicles. The lungs show a moderate number of small hemorrhages. Joint fluid clear.

November 6, cultures from the wall of the appendix wall yield many green colonies of streptococci and a few colonies of colon bacilli; from the blood, moderate number of green-producing streptococci and a few colon bacilli.

Sections of the appendix show marked hemorrhage chiefly in the spaces between the lymph follicles, but also in the latter. The nuclei of the fixed

cells here stain poorly and large numbers of streptococci and a few bacilli are present.

Rabbit 860.—Injected intravenously on November 3, with the growth from 30 c.c. of ascites dextrose broth inoculated with the pus obtained from the tonsil forty-eight hours after the operation and containing streptococci and colon bacilli.

November 4, found dead. Marked hemorrhages of the appendix; desquamation of the mucous membrane; the lumen free from fecal material. Moderate number of hemorrhages at ileocecal junction and a number of small areas in the lymphoid structures of the small intestines. The lymph gland at the root of the appendix is hemorrhagic, while those in the mesentery appear normal. No hemorrhages in stomach or duodenum. Gall-bladder, pancreas, heart valves, skeletal muscles, myocardium, kidneys, adrenals, eyes, brain, and thyroid show no hemorrhages. Liver is gray and opaque.

On November 5 the blood agar plates from the blood show a moderate number of green-producing streptococci and colon bacilli. The peritoneal coat overlying the appendix shows eight colonies of streptococci and one colony of colon bacilli. Sections show marked hemorrhage in the submucosa and in the lymph follicles. The nuclei of the tissue cells are fragmented and stain poorly, while stains for bacteria show many streptococci and some bacilli resembling colon bacilli.

Rabbit 862.—Injected on November 4 with the growth from 45 c.c. ascites dextrose broth of the streptococcus isolated from the appendix in Rabbit 847, which was injected with the mixture of the streptococcus and colon bacillus from the lumen of the appendix.

November 5, found dead, body warm. Marked hemorrhages in the appendix and in two Peyer's patches. Moderate number of smaller subperitoneal and submucous hemorrhages throughout the small and large intestine, most marked at the ileocecal junction. The lumen of the appendix is free from fecal material except for 1 cm., and contains a large amount of brownish mucus in which is a great quantity of desquamated material. The contents of the small intestines give a strong Weber test for blood. One large subcutaneous hemorrhage over right shoulder and a few hemorrhages in the heart valve; no other gross lesions.

On November 6 blood agar plates and ascites dextrose agar plates from the emulsion of the wall of the appendix show countless numbers of slightly green-producing streptococci and only forty colonies of colon bacilli. Cultures in ascites dextrose broth give similar results. The blood shows a pure culture of streptococcus. The joint fluid shows a few colonies of streptococci. Sections of the appendix show the hemorrhages are most marked in the lymph follicles, submucosa, and subperitoneum. The mucous membrane over these areas is absent. The nuclei of the fixed cells, in and adjacent to the areas which contain large numbers of streptococci, stain poorly. In several areas, the capillaries adjacent to areas of hemorrhage are plugged with streptococci and leukocytes.

Rabbit 865.—Injected intravenously on November 4 with the growth from 30 c.c. of the streptococcus isolated in pure form from the peritoneal coat of the appendix, now in the second culture.

November 6, seems quite well; chloroformed. Moderate number of fading hemorrhages in the appendix. No other gross lesions.

On November 10, cultures from the blood yield pure culture of green-producing streptococcus, from the joint fluid a few small slightly green colonies of streptococci.

Rabbit 880.—Injected intravenously November 6 with the growth from 15 c.c. ascites dextrose broth of the streptococcus isolated from the tonsil.

November 7, found dead, body warm. Appendix shows one large subperitoneal hemorrhage and numerous punctate hemorrhages in the submucosa; it contains a large amount of very turbid mucus but no fecal material. Moderate number of small punctate hemorrhages throughout the small intestine, particularly of Peyer's patches. Marked hemorrhages in the duodenum and cardiac end of stomach. The hemorrhages in the duodenum are distributed in a circular area around the ampulla of Vater. Joint fluids clear, no hemorrhages in skin. No other gross lesions.

On Novemebr 10 cultures from appendix yield many colonies of green-producing streptococci and a few colon bacilli; the blood yields a pure culture of streptococcus. Sections of the appendix show marked hemorrhage and necrosis of the submucosa and lymph follicles.

Rabbit 883.—Injected intravenously on November 7 with the growth from 45 c.c. of ascites dextrose broth of the streptococcus isolated from the blood in Rabbit 858, which was injected with the streptococcus isolated from the human appendix.

November 8, very ill; chloroformed. Moderate number of punctate hemorrhages in the appendix; the lumen contains a large amount of turbid, flaky mucus, but no fecal material. The lymph glands draining the appendix are hemorrhagic. Marked hemorrhages in the mucous membrane in the stomach with beginning ulceration; a few small hemorrhages in the duodenum; six subperitoneal hemorrhages in the gall-bladder, the largest, which is at the fundus, is surrounded by an edematous area of infection. No other gross lesions. Joint fluids clear.

On November 10 cultures from blood and area of infection in gall-bladder show large number of green-producing streptococci; joint fluid gives a few, while the bile is sterile.

CASE 190.—Acute gangrenous appendicitis in a man, 34 years of age. No history of any associated infection of the throat. On November 9, four days after the symptoms began, a swollen, gangrenous appendix was removed by Dr. Lewis. The lumen is free from fecal material, but contains a rather large amount of bloody pus without foul odor. Smears show a large number of gram-staining diplococci and bacilli resembling colon or fusiform bacilli.

On November 10 cultures on blood agar and ascites dextrose agar plates of the emulsion from the wall show mostly hemolytic colon bacilli and a few grayish colonies of a streptococcus which do not affect the blood in the plates. The peritoneal coat is sterile.

In animal experiments the intravenous injection of the bacteria directly from the emulsion of the appendix, chiefly streptococci, mixed aerobic cultures of colon bacilli and streptococci, and anaerobic cultures of these, containing in addition fusiform bacilli, produced lesions in the appendix almost exclusively in all of five rabbits. The organisms injected (except the fusiform bacillus) were demonstrated in the appendix either in sections or by cultures in the animals after death.

CASE 191.—Typical acute appendicitis in a woman, 45 years old, who has subject to tonsillitis and appendicitis, but this attack began without associated tonsillitis or other infection. A much swollen, almost black, perforated appendix was removed November 10 by Dr. Davis, thirty-six hours after the attack began. The patient died of general peritonitis three days later. The lumen

contains a small amount of bloody, foul-smelling pus and two fecal concretions. The mucous membrane is gangrenous and opposite the concretions the wall is perforated. The mesentery contains a large amount of fat and numerous hemorrhages. Smears from the fluid in the mesentery show gram-staining cocci and diplococci and a few gram-negative bacilli. The pus from the appendix shows gram-negative bacilli, resembling colon bacilli and fusiform bacilli, and a moderate number of gram-staining cocci.

On November 12 cultures from the pus within the appendix show colon bacilli, staphylococci, and an unidentified, small gram-negative bacillus; the wall of the appendix gives many gas bacilli, a few streptococci, a few colon bacilli, fusiform bacilli, and staphylococci; the peritoneal coat and edematous mesentery, many moderately hemolysing streptococci, a few gas bacilli and fusiform bacilli, and the staphylococcus aureus (Table 1). The cultures from the crypts of the tonsils show a predominating number of hemolytic streptococci, but also the streptococcus viridans and a moderate number of the staphylococcus aureus. The blood agar plates made from the ascites dextrose broth cultures inoculated with the material from the tonsils show a moderate number of colonies of streptococci indistinguishable from those isolated from the edematous mesentery.

Sections of the appendix show marked hemorrhage, leukocytic infiltration, and in areas necrosis in all the layers. The leukocytic infiltration is most marked surrounding the areas of necrosis in lymphoid tissue in the submucosa and in the peritoneal coat. The mucous membrane is absent. Gram-Weigert stains show many diplococci in chains together with single cocci, and, in addition, the superficial layers show a large number of bacilli resembling fusiform bacilli.

In the animal experiments the intravenous injection of the original cultures from the wall of the appendix and edematous fluid, containing mixtures of colon bacilli, streptococci, and a few staphylococci, failed to produce lesions in the appendix in three rabbits. The pure culture of slightly hemolysing streptococcus in the second culture isolated from the edematous mesentery also failed to produce appendicitis, but, instead, produced suppurative arthritis in four rabbits. On the other hand, the original culture from the tonsils, containing streptococci and staphylococci, produced distinct lesions in the appendix in two rabbits. The hemorrhages in the appendix seem to have been due in both instances to the staphylococcus, as shown by the cultures from the appendix. (See Rabbit 911.)

Rabbit 911.—Injected intravenously on November 12 with the growth from 45 c.c. of ascites dextrose broth inoculated with the material from the tonsils containing streptococcus and staphylococcus.

November 13, found dead. Numerous punctate hemorrhages in the mucous membrane and subperitoneum of the appendix. Joint fluid slightly turbid and moderate number of hemorrhages about knee joints. No other gross lesions.

November 14, blood agar plates, inoculated with the bloody fluid in the wall of the appendix, show large numbers of hemolysing colonies of staphylococci and a few streptococci; from the blood and joints, a large number of slightly hemolysing streptococci and a few staphylococci; from a bile, a few colonies of hemolysing staphylococci.

Rabbit 916.—Injected intravenously on November 13 with the growth from 10 c.c. of serum tissue broth of slightly hemolysing streptococci isolated from the edematous mesentery (second culture).

November 14, seems sick; chloroformed. No gross lesions except turbid joint fluid and periarticular hemorrhages.

November 16, blood agar plates inoculated with the blood and joint fluid show fifty colonies and four colonies, respectively, of a feebly hemolytic streptococcus.

CASE 199.—Typical, acute appendicitis in a girl, 13 years old. The attack came on late in the course of a severe Vincent's angina with cervical adenitis. Smears from the throat (Dr. Moody)) showed a large number of streptococci and fusiform bacilli. A gangrenous, foul smelling appendix was removed on November 15 by Dr. Halstead, seventy-two hours after the symptoms began. The appendix is much swollen, edematous, and dark red. The tissues are friable. The lumen contains one fecal concretion about the size of a navy-bean and a small amount of foul smelling, bloody pus. Mucous membrane is necrotic and ulcerated in places. Smears of the pus from the emulsion of the wall of the appendix and of the edematous peritoneal fluid show what appear to be streptococci, fusiform bacilli, and colon bacilli.

On November 17, the cultures in ascites dextrose broth of the emulsions from the wall, peritoneal coat, and edematous mesentery show colon bacilli, streptococci, fusiform bacilli, and spirilla. The cultures have a characteristic, foul odor. Blood agar and ascites dextrose agar plates show green-producing streptococci and colon bacilli from all. The proportion of streptococcus colonies increases as the cultures are made from the contents outward. The blood agar plate cultures from the tonsils show approximately an equal number of hemolysing and green-producing streptococci and a few staphylococci. Anaerobic cultures in ascites dextrose broth and on blood agar slants show streptococci in rather long chains and clumps and large numbers of fusiform bacilli.

Sections of the appendix show marked hemorrhages and leukocytic infiltration, particularly marked in the submucosa, subperitoneum, and lymphoid structures, while the muscular layer is relatively free. In many areas, the nuclei fail to stain. Gram-Weigert stains show large numbers of diplococci and chains, which appear in pure culture in the deeper layers and surrounding the areas of necrosis, while in the more superficial layers and in the center of the necrotic areas a large number of fusiform bacilli are also found.

In the animal experiments the intravenous injection of the primary aerobic and anaerobic cultures from the tonsil in ascites dextrose broth, containing streptococci, was followed by small hemorrhages in the appendices in all of the rabbits injected. In the second culture, the appendix was not affected but arthritis developed. The anaerobic cultures from the tonsil in ascites dextrose broth, containing streptococci and fusiform bacilli, produced lesions in the appendix and arthritis, while the anaerobic culture on blood agar produced only arthritis and hemorrhages in muscles. The primary aerobic cultures of the edematous mesentery and appendix, containing streptococci and colon bacilli, produced slight lesions of the appendices of two animals. The cultures, in which sterile tissue was added and which contained the fusiform bacillus in addition, and also those containing streptococcus and fusiform bacillus without colon bacillus produced marked lesions of the appendix in all but one of the rabbits, In the second culture, the affinity for the appendix was lost.

Rabbit 928.—Injected intravenously on November 17 with the growth from 30 c.c. ascites dextrose tissue broth containing streptococci, colon bacilli, and fusiform bacilli.

On November 18 animal was very ill; chloroformed, and examined at once. Moderate number of subperitoneal, circumscribed hemorrhages in the appendix.

Two small hemorrhages in the gall-bladder, one small hemorrhage in the ileum, a few under the skin in the muscles and kidney. Joint fluid clear.

November 19, aerobic blood agar plates from the blood show thirty-two colonies of colon bacilli and six colonies of a distinctly green-producing streptococcus. Hemorrhagic fluid under the peritoneum, in the appendix, from the skin and the joint fluid show no growth. One small hemorrhagic area in the appendix ground up in a mortar shows five colonies of green-producing streptococci, while another shows both streptococci and colon bacilli. The anaerobic cultures from the hemorrhagic area in the skin remain sterile while those from the lesions in the appendix give colon bacilli, fusiform bacilli, and streptococci.

Sections of the appendix show marked extravasation of red blood corpuscles and areas where the cells stain poorly. These contain a moderate number of streptococci and bacilli. Some of the latter resemble fusiform bacilli.

Rabbit 932.—Injected intravenously on November 18 with growth from 8 c.c. ascites dextrose tissue broth from the mesentery, containing streptococci, colon bacilli, and fusiform bacilli.

November 19, found dead. Large number of small punctate hemorrhages and four larger hemorrhages in the mucous membrane of the appendix; a few in the small intestines and at the ileocecal valve; two subperitoneal hemorrhages over the gall-bladder; and a few subpleural hemorrhages.

On November 21 cultures from the blood show almost a pure growth of green-producing streptococci and a few colonies of colon bacilli. The bile shows colon bacilli only, the hemorrhagic area in the appendix chiefly green-producing streptococi but some colon bacilli also. The fusiform bacillus is not found in the anaerobic cultures.

Rabbit 937.—Injected intravenously on November 19 with the growth from one anaerobic blood agar slant, inoculated with the emulsion from the mesentery containing streptococci and fusiform bacilli.

November 20, found dead. Large number of subperitoneal and submucous hemorrhages in the appendix, an occasional subperitoneal hemorrhage over the small intestine, and a large number of very small hemorrhages in the mucous membrane of the duodenum 2 cm. from the pyloric ring; a few small hemorrhages in the limbus of the left eye. No other gross lesions.

Smears from the emulsion of the peritoneal coat and hemorrhage in duodenum are negative; of the hemorrhagic wall of the appendix, show gram-positive and gram-negative bacilli and diplococci; from the hemorrhage in the eye, fusiform bacilli and diplococci.

November 22, cultures from the blood show streptococci, fusiform bacilli, and colon bacilli; from the duodenum, colon bacilli and streptococci; from the appendix, streptococci, fusiform bacilli, and colon bacilli; from the hemorrhage in the eye, colon bacilli and fusiform bacilli.

Sections of the appendix show a number of large and small hemorrhages and areas of necrosis in the connective tissue between and in the lymph follicles and submucosa. A large number of streptococci and fusiform bacilli are found in these areas. Sections of the duodenum show a moderate number of streptococci but no fusiform bacilli.

CASE 203.—A mild attack of appendicitis in a man. 19 years old. No history of tonsillitis or other associated infection; has not felt just right for two weeks and has lost twenty pounds in weight. A hyperemic and somewhat swollen appendix was removed on November 21, by Dr. Bevan, one week after the acute symptoms began. The lumen is free from fecal concretions and constricting bands and contains a small amount of bloody pus without odor; the

mucous membrane is hemorrhagic over two small areas and markedly congested everywhere.

On November 24, the cultures from the lumen, the wall, and peritoneal surface of the appendix show large numbers of hemolytic colon bacilli and a streptococcus which produces small, grayish, glistening, non-adherent colonies on blood agar plates, the peritoneal coat giving in addition a diphtheroid bacillus. Cultures from the right tonsil, which was swollen, showed the usual hemolysing and green-producing streptococci and a few colonies of staphylococci. Sections of the appendix show slight hemorrhages in the lymph follicles, submucosa, and just beneath the peritoneum. There are little leukocytic infiltration and no bacteria.

In the animal experiments the intravenous injection of the colon bacillus isolated from the appendiceal wall showed a pronounced affinity for the appendix and produced severe submucous and subperitoneal hemorrhages in two rabbits together with similar, but much less marked, lesions in the lymphoid structures of the intestines, and a few hemorrhages in the heart and skeletal muscles. In the second culture, this affinity was already much less marked (five rabbits), while in the fourth culture, twelve days later, the affinity had entirely disappeared. The mixture of colon bacilli and streptococci isolated from the peritoneal coat showed hemorrhages in the appendix and heart valves in one rabbit while the diphtheroid bacillus from the peritoneal coat failed to produce lesions anywhere in two rabbits. The mixed culture of the hemolytic and green-producing streptococci from the tonsil produced small hemorrhages in the appendix and arthritis in one rabbit, while the other showed hemorrhages in the tricuspid valve.

CASE 205.—Acute appendicitis in a man, 27 years old. No history of a sore throat. On November 25, fifty hours after the attack began, a markedly hyperemic and edematous appendix was removed by Dr. Phemister. A thick deposit of fibrin is found along the base and the mesentery; the lumen is free from fecal material and contains a small amount of bloody, odorless pus; the mucous membrane is hemorrhagic and in areas necrotic but there is no perforation. Smears from the pus in the appendix show a large number of gram-negative bacilli while those of the fibrin show streptococci only. The patient had fever for a number of days following the operation but made a good recovery.

Cultures from the pus show 5,400 colonies of colon bacilli; the emulsion of the wall, 680 colonies of a distinctly green-producing streptococcus and 14 colonies of colon bacilli; the peritoneal coat, 2,200 colonies of streptococci and 9 colonies of colon bacilli; and the fibrin, countless numbers of streptococci and 65 colonies of colon bacilli.

November 28, cultures which were made (November 27) from the pus expressed from the pockets in the tonsils and from the reddened, infected gums around a badly decayed, loose tooth yield chiefly short-chained streptococci in ascites dextrose broth; on blood agar, there is a great preponderance of green-producing colonies of streptococci. Sections of the appendix show marked hemorrhage, particularly just outside the circular muscle fibers. The mucous membrane and peritoneal coat are infiltrated with red blood corpuscles and leukocytes. Gram-Weigert stains for bacteria show diplococci and streptococci which are most numerous in the peritoneal coat and adjacent to the zone of hemorrhage. In the mucous membrane, a few bacilli are found.

In the animal experiments the intravenous injection of the streptococci in ascites dextrose broth from the tonsils and tooth and from the peritoneal coat, as well as the mixture of the colon bacillus and streptococcus from the wall

of the appendix, produced hemorrhages in the appendix in all but one of seven rabbits. The colon bacillus alone produced hemorrhages in the intestine but not in the appendix.

CASE 210.—Acute appendicitis in woman 19 years of age, following an attack of sore throat. On November 30, twelve hours after the symptoms of appendicitis began, a hyperemic and swollen appendix, 8 cm. in length, was removed by Dr. C. B. Davis. The lumen contains a moderate amount of bloody pus; there are no concretions or constrictions; the mucous membrane is hemorrhagic throughout. Smears from the pus show a large number of gram-staining diplococci and short chains and a few bacilli resembling colon and fusiform bacilli.

The cultures from the pus yield streptococci, colon bacilli, the bacillus welchii, and an unidentified bacillus; from the wall, colon bacilli, streptococci, the bacillus welchii and a diphtheroid-like streptococcus. The pus from the lumen in human serum gives many streptococci and a few fusiform and colon bacilli; in horse serum, there develops an almost pure culture of streptococci. Blood agar plates show that the streptococci from all three places in the appendix produce green colonies.

December 2, the cultures from the small amount of pus, expressed from the inflamed tonsils, in ascites dextrose tissue broth show short-chained streptococci and fusiform bacilli. Blood agar plates show a large preponderance of green-producing streptococci. Sections of the appendix show marked hemorrhage and leukocytic infiltration, particularly of the mucosa, submucosa, and the lymph follicles.

In the animal experiments the original cultures in ascites dextrose broth, containing a mixture of colon bacilli and streptococci from the lumen and wall of the appendix, the pure culture of the streptococcus from the peritoneal coat and the mixture of streptococcus and fusiform bacillus from the tonsil produced pronounced lesions in the appendix in four of six rabbits. The aerobic cultures from the tonsil, containing streptococci only, produced no lesions. The streptococcus from the lumen of the appendix, after one animal passage, produced marked hemorrhages in the gall-bladder, but no other lesions.

CASE 212.—Acute appendicitis in a medical student, 22 years old. Symptoms referable to the appendix began on the sixth day of an attack of severe tonsillitis, from which, however, he had recovered sufficiently to attend class late in the afternoon. The symptoms of appendicitis began that night. On December 3, twelve hours later, a markedly edematous and inflamed appendix was removed by Dr. C. B. Davis. The swelling is most marked near the tip opposite a cluster of fecal concretions; there is a rather thick fibrinous deposit; the mucous membrane opposite the concretions is markedly hemorrhagic and necrotic while the rest shows numerous small hemorrhages; the pus in the appendix is odorless.

The aerobic cultures from the pus within the appendix show a large number of the bacillus pyocyaneus, the bacillus coli, and streptococci, while the emulsion of a portion of the wall, from the peritoneal coat and edematous mesentery, after a thorough washing, shows a large number of the bacillus pyocyaneus and streptococci. The anaerobic cultures on blood agar have no odor and do not contain fusiform bacilli. The cultures from the tonsil, made December 3 at the time of the operation, show mostly green-producing streptococci and probably pneumococci and a few hemolytic streptococci. The streptococcus from the appendix and the green-producing streptococcus from the tonsil appear identical.

December 10, tonsils are somewhat red but do not contain cheesy material or pus in crypts. December 11, blood agar plates from the tonsil show almost pure culture of very small hemolytic colonies of streptococci and short chains in ascites dextrose broth.

Sections of appendix, opposite the fecal concretions, show marked leukocytic infiltration especially of the lymphoid tissue, submucosa, and peritoneal coat. The latter is covered with a layer of fibrin containing bacilli and streptococci. The mucous membrane is absent. Bacilli and streptococci are found throughout the wall. The latter appear in masses especially in the subperitoneal layer, where there are also three thrombosed blood vessels in one of which a few gram-positive diplococci can be seen (see photomicrographs).

In the animal experiments the intravenous injection of the primary aerobic culture from the tonsil, made at the time of the operation, and the second culture of the streptococcus from the edematous mesentery produced marked hemorrhages in the appendix in Rabbits 949, 992, and 993. The anaerobic culture of the former and the third subculture of the latter entirely failed to produce lesions in the appendix. The anaerobic cultures from the tonsil produced arthritis. The pure culture of the bacillus pyocyaneus showed no predilection for the appendix, but produced many small punctate hemorrhages throughout the intestinal tract and in the lung. The streptococci from the tonsil seven days later failed to produce appendicitis or other lesions (Rabbits 991 and 999).

RESULTS OF THE CULTURES

Cultures from the appendix have been made in fourteen cases of acute appendicitis and six cases of chronic appendicitis (Table 1).

Fecal concretions were present in seven; in the rest, no local, mechanical factors could be made out. Streptococci, usually in predominating numbers, were isolated from the tissues of the appendix in seventeen cases. The colon bacillus was found in pure cultures in the pus within the lumen in six cases and in predominating numbers, but with streptococci or other organisms in the rest. The results of the cultures from the wall, after thorough washing, showed that here the chief bacteria were streptococci. The fusiform bacillus was isolated from the wall of the appendix in three cases (Cases 191, 199, and 96); other anaerobic bacilli and spirilla were found in some cases, but were not identified; the bacillus welchii in two; a diphtheroid bacillus in two; the staphylococcus aureus in one; the bacillus pyocyaneus in one, and unidentified cocci and bacilli in two. The strains of streptococci which were found to have an affinity for the appendix formed short chains, much acid and a diffuse turbidity in ascites dextrose broth, but no clumps and, with but two exceptions, produced a moderate amount of green on blood agar plates. After animal passage they produced more green on blood agar and became more like pneumococci, several strains acquiring capsules. They were larger than the streptococcus

viridans obtained from the blood in chronic endocarditis and the colonies, with one exception, were non-adherent. In a number of cases, two types of non-hemolyzing colonies were obtained; one producing green colonies, and the other non-adherent, small, grayish colonies (Str. fecalis). The latter, as well as the strains producing hemolysis, showed no special affinity for the animal appendix.

In the peritoneal coat and mesentery there were less colon bacilli in proportion to streptococci than in the other coats, and sometimes they were entirely absent. Thus, the cultures from the contents of the appendix of Case 205 showed 3,400 colonies of colon bacilli, pure; the wall, 680 colonies of streptococci and 14 colonies of colon bacilli; the peritoneal coat, 2,200 colonies of streptococci and 9 colonies of colon bacilli; while the edematous mesentery and fibrin showed countless numbers of streptococci and 65 colonies of colon bacilli.

These results are in accord with the sections and indicate that reliable differential cultures can be made of the various tissues in appendicitis. This is further borne out by the fact that the peritoneal coat in four cases remained sterile when the more superficial layers contained organisms, and that cultures remained sterile in three normal appendices, in one of six appendices with chronic appendicitis, and in three obliterated appendices.

The character of the bacterial flora of the tonsils in the different cases was not characteristic. Streptococci were found in all; in nearly all, the green-producing variety predominated. The staphylococcus aureus was found in rather large numbers in three; fusiform bacilli were in large numbers in two; and the colon bacillus was found in one.

MICROSCOPIC ANATOMY OF THE APPENDIX

The areas of hemorrhage which are found at the end of twenty-four hours are usually in the submucosa, lymph follicles, and subperitoneal region. At this time there is not much leukocytic infiltration. In five of the appendices from young individuals in which the lesions were relatively slight, the distribution of the hemorrhages and other lesions were strikingly similar. In the experimental lesions, the hemorrhages usually had started to fade and the mucous membrane might be eroded at the end of forty-eight hours. The ulceration of the mucous membrane usually begins directly over the edematous lymph follicles with necrosis and leukocytic infiltration. Here, the nuclei of the fixed tissue cells fail to take the stain, or are fragmented and granular. It is in

these areas, as well as in the areas of hemorrhage, that masses of the injected streptococci are found and where the adjacent normal tissues are free from bacteria. In many instances in which mixtures of streptococci and colon bacilli were injected, the former were usually found in predominating numbers and, in some instances, to the exclusion of the latter. Similar conditions have been found in a number of human appendices. In some instances, capillaries in the appendices of rabbits adjacent to an area of hemorrhage have been found plugged with streptococci (see plates). The organisms found have been shown to be alive in many instances. Very large numbers may be found in the lesions eighteen hours after injection when control cultures of the uninvolved portions of the appendix, other normal tissues, and the blood were sterile or contained only few organisms. Moreover, sections of parts in which no macroscopic evidence of lesions could be made out usually failed to show either lesions or bacteria.

Owing to the larger size of the lumen of the appendix, strangulation, with its consequences, has not been observed. In several instances, however, the lesions were so pronounced that the lesions appeared almost gangrenous in twenty-four and forty-eight hours after injection. Following the injection, the hemorrhages and other lesions showed no tendency to localize around chronic coccidial lesions.

RESULTS OF INTRAVENOUS INJECTIONS

A summary of the results from intravenous injection in rabbits of the isolated strains is given in Table 2.

The elective affinity for the appendix of the streptococci in pure culture or in mixture with fusiform bacilli, isolated both from the tonsils at the time of, or soon after, the attack and from the appendix, is striking. Thus, the tonsillar strains produced appendicitis in nineteen of twenty-nine, the appendix strains in twenty-two of thirty, a total of forty-one of fifty-nine. After cultivation on artificial media for a short time the elective affinity is soon lost, and strains isolated from the tonsils some time after appendectomy also appear without elective affinity.

The results following injection of mixtures of streptococci and colon bacilli are similar, altho the tendency to produce lesions in the intestine and gall-bladder is much greater. Thus, of thirty-one rabbits injected, twenty-six showed lesions in the appendix, four of the gall-bladder and thirteen of the intestines, in contrast to one and four,

respectively, of fifty-nine rabbits injected with the streptococcus only. On the other hand, the tendency to produce lesions in the joints and heart valves by the streptococcus, when injected with the colon bacillus, is strikingly less. Arthritis occurred only in two and endocarditis in

TABLE 1

SUMMARY OF BACTERIOLOGICAL STUDY OF APPENDICITIS

Case	Age	Operator	Diagnosis	Duration of Symptoms Before Operation	Organisms Isolated
					Contents of Appendix
128	12	D. C. Balfour	Recurrent appendicitis and tonsillitis	3 days	Colon bacilli .....
182	12	C. B. Davis...	Acute appendicitis	12 hours	Colon bacillus and streptococcus
184	17	C. B. Davis...	Acute gangrenous appendicitis	4 days	Chiefly colon bacilli and a few streptococci
190	34	D. D. Lewis...	Acute gangrenous appendicitis	4 days	Colon bacillus and streptococcus
191	45	C. B. Davis...	Acute gangrenous appendicitis with perforation	2 days	Colon bacillus, a small, unidentified, gram-negative bacillus, and staphylococcus
199	13	A. E. Halstead	Acute gangrenous fetid appendicitis and Vincent's angina	2 days	Colon bacillus and a few streptococci
203	19	A. D. Bevan...	Acute appendicitis	7 days	Colon bacillus and streptococcus
205	27	D. B. Phemister	Acute appendicitis	2 days	3,400 colonies of colon bacillus
210	19	C. B. Davis...	Acute appendicitis	12 hours	Streptococcus, colon bacillus, B. welchii, and an unidentified coccus
211	19	C. B. Davis...	Acute (mild) appendicitis...	2 days	Colon bacillus and B. welchii
212	22	C. B. Davis...	Acute appendicitis	12 hours	B. pyocyaneous, colon bacillus, and streptococcus
96	12	W. J. Mayo...	Acute recurring appendicitis and tonsillitis	12 hours	Colon bacillus .....
84	36	D. C. Balfour	Acute gangrenous, perforating appendicitis	2 days	Colon bacillus and streptococcus
73	50	E. S. Judd....	Chronic appendicitis and cholecystitis	.....	Colon bacillus .....
72	..	E. S. Judd....	Chronic appendicitis	.....	.....
75	..	C. H. Mayo..	Chronic appendicitis	.....	.....
58	..	.....	Chronic cholecystitis appendicitis obliterans	.....	Colon bacillus .....
59	..	.....	Chronic cholecystitis, chronic appendicitis	.....	.....

one rabbit of thirty-one, whereas in thirty rabbits injected with streptococci one arthritis occurred in seven and endocarditis in six. These results are in entire accord with those of injections of pure cultures of the colon bacillus. Of eleven rabbits injected, six showed lesions in the appendix and six in the intestines.

The elective affinity for the appendix also disappears after one to three animal passages, producing now frequently less-marked lesions in the appendix, but more often lesions in the stomach, duodenum, and gall-bladder. Thus, of twenty rabbits injected only ten showed slight

TABLE 1.—*Continued*

SUMMARY OF BACTERIOLOGICAL STUDY OF APPENDICITIS

Organisms Isolated			Remarks
Wall of Appendix	Peritoneal Coat	Edematous Mesentery	
Colon bacilli and green-producing streptococci	Sterile	.....	Gland at base of appendix yields green-producing streptococcus only
Streptococcus and colon bacillus	Sterile		
Chiefly streptococci, also a few colonies of colon bacilli	3,600 colonies streptococci		
Colon bacillus, aerobic and anaerobic streptococci, and fusiform bacilli	Sterile		
B. welchii, a few streptococci, colon bacilli, fusiform bacilli, and staphylococci	Many streptococci, a few B. welchii, fusiform bacilli, and staphylococci	Many streptococci, a few B. welchii, a few colon bacilli, and a moderate number of staphylococci	Death 3 days after operation
Colon bacillus, streptococci, fusiform bacilli, and spirilla	Colon bacillus, streptococcus, fusiform bacillus, and spirilla	Colon bacillus, streptococcus, fusiform bacillus, and spirilla	Smears from edematous fluid show colon bacillus, streptococcus, large numbers of fusiform bacilli
Streptococcus and colon bacillus	Streptococcus and bacillus		
680 colonies of streptococci and 14 colonies of colon bacillus	2,200 colonies streptococcus and 9 colonies colon bacillus	Fibrin, countless numbers of streptococci and 65 colonies of B. coli	
Colon bacillus, streptococcus, B. welchii, and diphtheroid-like bacillus	Diphtheroid-like bacillus		
Streptococcus, colon bacillus, and B. welchii	Sterile		
B. pyocyaneous and streptococcus	B. pyocyaneous and streptococcus	Streptococcus and countless number of colonies of B. pyocyaneous	Edematous fluid in a serum culture yielded a pure culture of streptococcus
Streptococcus and fusiform bacillus			
Streptococcus and colon bacillus			
Streptococcus and colon bacillus			
.....	Streptococcus	.....	Interim operation, appendix thickened
Streptococcus and colon bacillus	.....	.....	Symptoms referable to stomach. Appendix thickened
Sterile			
Streptococcus and colon bacillus			

lesions in the appendix, ten hemorrhages in the stomach or duodenum, seven ulcers, and eight lesions in the gall-bladder. This is in sharp contrast to the result of immediate injections of the strains from the tonsil at the time of the attack and the appendix, when lesions in the stomach and duodenum were found only seven times and in the gall-bladder only once in fifty-nine rabbits.

TABLE 2

RESULTS FOLLOWING INTRAVENOUS INJECTIONS

Strains Injected	Number of Animals Injected	Number of Times Lesions Were Found in										
		Appendix	Stomach and Duodenum		Gall Bladder	Pancreas	Joints	Endocardium	Myocardium	Muscles	Kidney	Intestines
			Hemorrhages	Ulcers								
Streptococci in pure culture, or in mixture with fusiform bacilli, soon after isolation, from tonsil at time of attack	29	19	4	0	0	0	10	7	4	5	0	2
Streptococci in pure culture, or in mixture with fusiform bacilli, from the appendix soon after isolation	30	22	2	1	1	..	7	6	1	2	..	2
Streptococci from tonsil and from the appendix, after cultivation on artificial media for a short time, and from the tonsils some time subsequent to appendectomy	22	3	5	4	1	0	8	5	3	6	0	0
Streptococci and colon bacilli in mixed cultures from the tonsil or appendix soon after isolation	31	26	6	0	4	0	2	1	3	3	1	13
Streptococci from the tonsil or appendix after one or three animal passages	20	10	10	7	8	0	8	4	4	5	2	4
Pure cultures of the colon bacillus from the appendix, soon after isolation	11	6	3	0	0	0	0	1	2	2	0	6
Total	143	86	30	12	14	0	36	24	17	23	3	27

The relatively large number of times arthritis developed after injection of the streptococcus was due no doubt in part to mixtures of the hemolytic streptococcus and green-producing streptococcus from the tonsils and to the slightly hemolyzing strain which was found in the appendix in one of the cases (Case 191).

At times, it was possible to isolate from the rabbits after injection of mixtures green-producing streptococci from the appendix and hemolyzing streptococci from the joint fluid. The streptococci from the appendix, as well as from the tonsil, which show affinity for the appendix are of a relatively low grade of virulence They tend to disappear from the circulation unless very large doses are given, thereby affording opportunity to study their relation to various lesions produced by making cultures from the tissues involved. In Case 191, which died of peritonitis on the third day, moderately hemolyzing and relatively virulent streptococci were found, which on injection showed no affinity for the appendix, but a marked affinity for the joints. The streptococci isolated from the wall of the appendix in the three cases of chronic appendicitis and cholecystitis, which were identical with the strains from the gall-bladder in two of the cases, showed a tendency to infect simultaneously appendix, stomach, and gall-bladder when injected into rabbits. Thus, of eight rabbits injected, three showed lesions in the appendix, three in the gall-bladder, and three in the stomach. In none was the appendix involved to the exclusion of the gall-bladder or stomach.

The colon bacillus in most cases is to be regarded as a secondary invader because it is found both by cultures and in sections either in decreasing numbers in the tissue from the lumen outward, or is displaced entirely by the streptococci (Cases 128, 184, 191, 205, 210, 211, and 212), and because when injected into animals in pure culture it failed to infect the appendix, except in the case of the strains from Case 203. This accords with the results of Beaussenat cited by Adrian,[9] who was unable to produce appendicitis by intravenous injection of colon bacilli without injuring the mucous membrane.

In some instances in which mixtures of streptococci and colon bacilli were injected, the former were found in predominating numbers in the appendix as shown both by cultural and by microscopic examinations (Rabbits 798, 846, 847, 859, and 860). The bacillus pyocyaneus, found in the appendix in Case 212, and the diphtheroid bacillus, found

9. Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1901, 7, p. 407.

TABLE 3

LESIONS PRODUCED BY INTRAVENOUS INJECTION OF STRAINS ISOLATED FROM CASE 128

Animal	Date of Injection	Bacteria Injected	Amount in c.c. Injected	Date of Autopsy	Lesions in											Remarks
					Appendix	Stomach and Duodenum		Gall-Bladder	Pancreas	Joints	Endocardium	Myocardium	Muscles	Kidneys	Intestines	
						Hemorrhages	Ulcers									
Rabbit 686.....	Aug. 23	Streptococcus from tonsil, first culture	45	Aug. 24	++	+	0	0	0	+	0	0	0	0	0	Small hemorrhage just beneath the mucosa
Rabbit 689.....	Aug. 24	Streptococcus from tonsil, second subculture	45	Aug. 26	+	0	0	0	0	0	0	0	+	0	0	
Rabbit 692.....	Aug. 25	Same as Rabbit 686 after streptococcus kept in NaCl solution for 48 hours	20	Aug. 26	0	0	0	0	0	+	0	0	+	0	0	
Rabbit 693.....	Aug. 25	Same as Rabbit 686 after streptococcus kept in NaCl solution for 48 hours	20	Aug. 26	++	0	0	0	0	+	0	0	0	0	0	
Rabbit 702.....	Aug. 26	Streptococcus from tonsil, third subculture	50	Aug. 27	0	0	0	0	0	+	+	0	0	0	0	
Rabbit 703.....	Aug. 26	Streptococcus from tonsil, third subculture	75	Aug. 27	+	0	0	0	0	+	0	0	+	0	0	Sections of the appendix show hemorrhages in the mucosa, margin of lymph follicles, and under the peritoneal coat
Rabbit 687.....	Aug. 23	Colon bacillus from pus in appendix	75	Aug. 24	0	0	0	0	0	0	0	0	0	0	0	Overwhelming infection
Rabbit 704.....	Aug. 26	Colon bacillus from pus in appendix	60	Aug. 27	0	0	0	0	0	0	0	0	0	0	0	Overwhelming infection
Rabbit 705.....	Aug. 26	Streptococcus and colon bacillus from appendix	15	Aug. 27	++	0	0	0	0	0	0	+	0	0	0	
Rabbit 709.....	Aug. 28	Streptococcus from appendix	60	Aug. 31	++	0	0	0	0	+	0	0	0	0	0	
Rabbit 710.....	Aug. 28	Streptococcus from appendix	60	Aug. 29	++	+	0	0	0	0	+	0	0	0	0	
Rabbit 713.....	Aug. 28	Streptococcus from appendix	60	Aug. 31	++	0	0	0	0	+	0	0	0	0	0	
Rabbit 714.....	Aug. 28	Streptococcus from appendix	60	Aug. 29	++	0	0	0	0	0	0	0	0	0	0	
Rabbit 719.....	Sept. 1	Streptococcus from appendix after 1 animal passage	45	Sept. 3	0	+	+	0	0	+	0	0	0	0	0	
Rabbit 720.....	Sept. 1	Streptococcus from appendix after 1 animal passage	60	Sept. 2	0	++	0	0	0	0	+	0	0	0	0	
Rabbit 724.....	Sept. 3	Streptococcus from appendix after 2 animal passages	4.5	Sept. 5	0	++	+	+	0	++	+	+	+	0	0	
Dog 131	Sept. 4	Streptococcus from appendix after 2 animal passages	75	Sept. 5	0	++	+	0	0	0	0	0	0	0	+	
Rabbit 728.....	Sept. 6	Streptococcus from appendix after 3 animal passages	3	Sept. 8	+	++	+	0	0	+	0	+	+	0	0	
Guinea-pig 1234	Sept. 6	Streptococcus from appendix after 3 animal passages	2	Sept. 9	0	++	+	0	0	+	0	0	0	0	0	

TABLE 4

LESIONS PRODUCED BY INTRAVENOUS INJECTIONS OF STRAINS FROM CASE 212

Rabbit	Date of Injection	Bacteria Injected	Amount Injected	Date of Autopsy	Lesions in											Remarks
					Appendix	Stomach and Duodenum		Gall-Bladder	Pancreas	Joints	Endocardium	Myocardium	Muscles	Kidneys	Intestines	
						Hemorrhages	Ulcers									
	December			December												
749	4	Streptococcus from tonsil	45 c.c.	5	++	0	0	0	0	0	+	0	0	0	0	
751	4	B. pyocyaneous from mesentery	45 c.c.	5	0	0	+	0	0	0	0	0	0	0	++	Marked hemorrhage in lung
989	5	Anaerobic culture from tonsil	One slant	7	0	0	0	0	0	+	0	0	0	0	0	
992	5	Streptococcus from edematous fluid	15 c.c.	7	++	0	0	0	0	0	0	0	0	0	+	
993	5	Streptococcus from edematous fluid	5 c.c.	7	++	0	0	0	0	0	0	0	0	0	+	
995	7	Streptococcus from edematous fluid in third subculture	30 c.c.	8	0	0	0	0	0	0	0	0	0	0	0	
996	7	Streptococcus from edematous fluid in third subculture	30 c.c.	8	0	0	0	0	0	0	0	0	0	0	0	
991	10	Streptococcus from tonsil, 12/9/14	45 c.c.	11	0	0	0	0	0	0	+	0	0	0	0	
999	10	Streptococcus from tonsil, 12/9/14	30 c.c.	11	0	0	0	0	0	0	0	0	0	0	0	

TABLE 5

LESIONS PRODUCED BY INTRAVENOUS INJECTIONS OF STRAINS FROM CASE 184

Rabbit	Date of Injection	Bacteria Injected	Amount in c.c. Injected	Date of Autopsy	Lesions in										Remarks
					Appendix	Stomach and Duodenum		Gall-Bladder	Pancreas	Joints	Endocardium	Myocardium	Muscles	Kidneys	Intestines	
						Hemorrhages	Ulcers									
845	November 1	Streptococcus and colon bacillus from appendix	15	November 5	+++	0	0	0	0	0	0	0	0	0	+	
846	1	Streptococcus and colon bacillus from appendix	15	2	+++	0	0	0	0	0	0	0	0	0	+	Section of the appendix shows many streptococci, and a few colon bacilli in areas of hemorrhage
847	1	Streptococcus and colon bacillus from appendix	1.5	3	+++	0	0	0	0	0	0	0	0	0	0	
849	1	Streptococcus and colon bacillus from appendix	30	2	++++	0	0	0	0	0	0	0	0	0	+	
887	8	Streptococcus and colon bacillus from peritoneum	45	9	+++	0	0	0	0	0	0	0	0	0	+	
888	8	Streptococcus and colon bacillus from peritoneum	20	9	+++	0	0	0	0	0	0	0	0	0	0	
785	2	Streptococcus from peritoneal coat	30	3	+	0	0	0	0	0	0	0	0	0	0	
851	2	Streptococcus from peritoneal coat	30	4	+	0	0	0	++	0	0	0	0	0	0	
852	2	Streptococcus from peritoneal coat	15	5	+	0	0	0	0	0	0	0	0	0	0	
853	2	Streptococcus from peritoneal coat	15	3	++	0	0	0	0	0	+	0	0	0	0	
854	2	Streptococcus from peritoneal coat	30	3	+++	0	0	0	0	0	+	0	0	0	0	Sections of the appendix show many streptococci on areas of hemorrhage and necrosis.
855	2	Streptococcus from edematous peritoneal coat	30	4	0	0	0	0	0	0	0	0	0	0	0	Injection made into lumen of appendix
864	4	Streptococcus from peritoneal coat	60	6	++	0	0	0	0	0	0	0	0	0	0	
865	4	Streptococcus from peritoneal coat	30	6	++	0	0	0	0	0	0	0	0	0	0	
866	4	Streptococcus from peritoneal coat	30	6	++	0	0	+	0	0	0	0	0	0	0	
857	3	Streptococci and colon bacillus from tonsil	15	4	++++	+	0	0	0	0	0	0	0	0	+	Section of the appendix shows many streptococci and a few colon bacilli in areas of hemorrhage
858	3	Streptococci and colon bacillus from tonsil	15	4	++	+	0	0	0	0	0	0	0	0	+	

859	3	Streptococcus and colon bacillus from tonsil	30	4	++	0	0	0	0	0	+	0	0	0	0	Sections of the appendix show many streptococci in areas of hemorrhage
860	3	Streptococcus and colon bacillus from tonsil	30	4	++++	0	0	0	0	0	0	0	0	0	+	A few subcutaneous hemorrhages
890	8	Streptococci from tonsil	45	9	++	0	0	0	0	0	0	0	0	0	0	
877	6	Streptococcus from appendix after one animal passage	60	7	+	++	0	+	0	0	0	0	0	0	0	Streptococci in pure culture obtained from lesions
878	6	Streptococcus from appendix after one animal passage	45	8	0	0	0	+	0	++	+	+	+	++	0	
879	6	Streptococcus from appendix after one animal passage	60	7	+	0	0	0	0	0	0	0	0	0	+	Pleuritis and subcutaneous cellulitis
880	6	Streptococcus from appendix after one animal passage	4.5	7	+	+	+	0	0	0	0	0	0	0	0	Sections of the appendix show many streptococci in areas of hemorrhage
883	7	Streptococcus from appendix after one animal passage	45	8	+	+	+	+	0	0	0	0	0	0	+	
862	4	Streptococcus from appendix after one animal passage	45	5	+++	0	0	0	0	0	0	+	0	0	++	Sections of the appendix show many streptococci in areas of hemorrhage and necrosis
863	4	Streptococcus from appendix after one animal passage	30	5	+++	0	0	0	0	0	0	0	0	0	+	Sections of the appendix show many streptococci in areas of hemorrhage and necrosis
868	5	Streptococci from tonsil after one animal passage	10	7	+	0	0	0	0	0	0	0	0	0	0	
869	5	Streptococci from tonsil after one animal passage	10	7	+	0	0	0	0	0	0	0	0	0	0	
870	5	Streptococci from tonsil after one animal passage	10	7	0	0	0	0	0	0	0	0	0	0	0	Forty-eight-hour culture
875	6	Streptococci from appendix after two animal passages	10	8	0	0	0	+	0	+	0	0	0	+	0	
876	6	Streptococci from appendix after two animal passages	10	7	+	+	0	0	0	+	0	0	0	0	0	
892	9	Streptococci from appendix after three animal passages	15	11	0	0	0	++	0	0	0	0	+	0	0	
894	9	Streptococci from appendix after three animal passages	30	10	0	0	0	+	0	+	+	0	+	0	0	
889	8	Streptococci from peritoneum	60	9	0	0	0	0	0	0	0	0	0	0	0	Cultivated on Loeffler's serum for 4 days
881	7	Streptococci from tonsil...	45	9	0	0	0	+	0	+	+	+	+	0	0	Second tonsil culture, long chains
882	7	Streptococci from tonsil...	30	8	0	0	0	0	0	+	+	+	0	0	0	Second tonsil culture

TABLE 5.—*Continued*

LESIONS PRODUCED BY INTRAVENOUS INJECTIONS OF STRAINS FROM CASE 184

Rabbit	Date of Injection	Bacteria Injected	Amount in c.c. Injected	Date of Autopsy	Lesions in										Remarks
					Appendix	Stomach and Duodenum		Gall-Bladder	Pancreas	Joints	Endocardium	Myocardium	Muscles	Kidneys	Intestines	
						Hemorrhages	Ulcers									
891	November 9	Streptococci from tonsil and colon bacilli from appendix	15	November 11	0	0	0	0	0	0	0	0	0	0	0	Streptococci from second culture
893	9	Streptococci from tonsil and colon bacilli from appendix	30	10	0	0	0	0	0	0	0	0	0	0	0	Marked hemorrhages in both testicles. Many streptococci and a few colon bacilli
901	11	Streptococci from appendix in ascites dextrose broth 10 days	30	12	0	0	0	0	0	+	+	0	0	0	0	
829	8	Streptococci from tonsil after 8 days cultivation	30	9	+	0	0	0	0	0	0	0	0	0	0	
831	8	Streptococcus from appendix after 8 days cultivation	30	9	0	0	0	0	0	0	0	0	0	0	0	
885	11	Colon bacilli from appendix	30	9	0	+	0	0	0	0	0	0	0	0	+	Colon bacilli, pure culture
886	8	Colon bacilli from appendix	30	9	0	+	0	0	0	0	0	0	0	0	+	Colon bacilli, pure culture

in the peritoneal coat in Case 203, showed no special affinity for the appendix.

Three rabbits were injected directly into the appendix with strains shown to have affinity for the appendix when injected intravenously, but appendicitis did not develop.

In five of the cases (Cases 128, 199, 210, 212, and 96) the hematogenous origin is indicated by the clinical history as well as by the elective affinity for the appendix of the strains isolated from the tonsils and the appendix. In four cases (Cases 167, to be reported elsewhere, 184, 191, and 205) no history of an associated infection was apparent, but an unsuspected focus in the tonsils (or teeth) was found to contain, at the time of the attack, streptococci with an elective affinity for the appendices of rabbits. In two of the cases, the assumed primary focus was found four to twenty-five days later to contain streptococci which no longer had such affinity. In the other cases (Cases 190, 203, 211, 182, and 184) there was no history to lead one to suspect a hematogenous origin, but in all but one the appendix contained organisms which showed an affinity for the appendices of rabbits when injected intravenously. In two of these, the suspected foci did not contain streptococci which had an elective affinity for the appendix. In the rest no cultures were made from the tonsils.

GENERAL DISCUSSION

It must not be supposed that the infections of the appendices in the rabbits described in the foregoing are simply a part of wide-spread lesions due to the injections of large doses. The elective affinity for the appendix is shown, in some instances, when very small doses are injected. Not infrequently, the amount of involvement in the appendix is in proportion to the size of the dose. The relatively small number of organisms present in emulsions of small portions of the human appendices when injected intravenously localized in the appendices of the rabbits in two instances, thus showing that the affinity is not due to some peculiarity the organisms acquired in the cultures. It was found that emulsions of the appendix, especially when showing marked lesions and many bacteria, were exceedingly toxic, which made it necessary to separate the bacteria from the cells by centrifugation in order to demonstrate the localization of the bacteria in the appendix.

Very young and old rabbits are less prone to develop lesions in the appendix on intravenous injection than half-grown rabbits, and the lesions that so develop in the latter are more marked.

The statements of Aschoff and Ghon and Namba to the effect that if appendicitis commonly is embolic in origin it must be due to bacteria having elective affinity for the appendix would seem to correspond to the facts. The evidence in favor of the view that a focus of infection in the tonsil [or teeth] is primary and the infection in the appendix is secondary is strong. The reverse, namely, that the bacteria in the tonsil are brought there by the blood stream, hardly needs to be discussed, because the inflammation in the tonsils is most acute for some time previous to the attack of the appendicitis. In no case was there any localization in the tonsils in the animals. In the one case in which the colon bacillus was found in the tonsil, having presumably been carried there by the blood, the tonsil showed no noteworthy inflammation. The further fact that the strains both from the tonsil and appendix which showed an affinity for the appendix were different than the usual streptococcus normally present in the intestinal tract, which did not show such affinity, also speaks in favor of the view that the primary focus is in the tonsils [or teeth].

The mucous membrane of the appendix, either human or animal, often bulged markedly when the peritoneal coat was dissected away. The lumen of the inflamed appendix in the rabbit was almost invariably free from fecal contents, except at the base, containing usually mucus with flakes of partially digested mucous membrane, while the lumen of the appendix of a normal rabbit usually contained feces to the very tip. These observations indicate that the cause of the colicky pain in human appendicitis, especially when out of proportion to the actual lesion, is largely due to spasms of the muscles, caused most likely by a relative lack of oxygen and increased acidity, the result of local infection, as developed by Graham.[10]

SUMMARY

The results of the observations and experiments indicate that appendicitis, in the absence of foreign bodies, commonly is a hematogenous infection, secondary to some distant focus; that it develops when, for some reason or other, the organisms in the focus, usually streptococci, have acquired an elective affinity for the appendix and at the same time gain entrance into the circulation.

The results bear out my theory that a focus of infection is to be looked on, not only as the place of entrance of bacteria, but also as

10. Surg. Gynec. Obst., 1914, 19, p. 360.

the place where they may acquire the varying affinities necessary to infect distant organs and tissues.

From the results in the animals, it seems, as emphasized also by Heyde, Aschoff, and others, that appendicitis is a serious disease, not so much on account of the virulence of the infecting micro-organisms as on account of the anatomy of the appendix favoring strangulation and thus the growth especially of facultative and strict anaerobes.

The importance of thorough search for and removal of possible foci of infection from which appendicitis may originate must be emphasized.

Finally, it may be pointed out that the frequent occurrence of appendicitis, at times almost in epidemic form when throat infections are particularly prevalent, now is easily understood.

EXPLANATION OF PLATES 12 TO 16

PLATE 12

Fig. 1.—Appendicitis in rabbit, twenty-four hours after intravenous injection of a streptococcus from a degenerating colloid goiter in man. Note the hemorrhages and edema in the appendix and the absence of lesions in the cecum. Reflected light.

Fig. 2.—Ulcerative appendicitis in rabbit, five days after intravenous injection of streptococci from rheumatism after one animal passage. Note the marked edema and ulceration of the mucous membrane. Reflected light.

Fig. 3.—Hemorrhagic appendicitis in rabbit, twenty-four hours after intravenous injection of streptococci from tonsil in a case of appendicitis in man (Case 184). Note the marked circumscribed hemorrhages and swelling of the mucous membrane in the appendix and their absence in cecum. Transmitted light.

Fig. 4.—Hemorrhagic appendicitis in rabbit, twenty-four hours after intravenous injection of colon bacilli from the appendix in human appendicitis (Case 203). Note the circumscribed hemorrhages in the appendix and their absence in the cecum. Transmitted light.

Fig. 5.—Appendix of rabbit showing spontaneous retention cysts of the mucous glands. Reflected light.

Fig. 6.—Marked hemorrhages in the duodenum in rabbit, which also had marked hemorrhages in the appendix, twenty-four hours after intravenous injection of colon bacilli from human appendicitis (Case 203).

PLATE 13

Fig. 7.—Small intestine of rabbit showing a swollen, hemorrhagic Peyer's patch twenty-four hours after intravenous injection of a streptococcus from the appendix in human appendicitis after one animal passage (Case 184). The rabbit also had marked appendicitis.

Fig. 8.—Hemorrhages in the appendix and duodenum and hemorrhages and ulceration in the stomach in rabbit, forty-eight hours after intravenous injection of a streptococcus from the appendix in human appendicitis, after three animal passages (Case 128).

Fig. 9.—Appendix removed twelve hours after the onset of acute appendicitis in a young man. Note the necrosis and hemorrhages of the lymph follicle and the marked infiltration throughout the wall. × 50.

Fig. 10.—Diplococci in the peritoneal coat of appendix shown in Figure 9.

PLATE 14

Fig. 11.—Appendicitis in rabbit, twenty hours after intravenous injection of streptococci from the appendix in human appendicitis. Note the hemorrhages and necrosis, particularly of the lymph follicle, and the infiltration and sloughing of the mucous membrane. × 600.

Fig. 12.—Streptococci in the submucosa of the appendix shown in Fig. 11. × 1200.

Fig. 13.—Streptococci in the lymph follicle of the appendix shown in Fig. 11. × 1200.

Fig. 14.—Appendicitis in rabbit twenty-four hours after intravenous injection of a mixed culture of streptococci and colon bacilli from the appendix in human appendicitis. Note the marked hemorrhages and necrosis in lymph follicle, infiltration of the submucosa, and ulceration of the mucosa.

Fig. 15.—Streptococci and a few colon bacilli in the appendix shown in Fig. 14. × 1200.

PLATE 15

Fig. 16.—Human gangrenous and fetid appendicitis, following Vincent's angina. Section of the appendix shows marked necrosis and leukocytic infiltration throughout. × 45.

Fig. 17.—Streptococci and fusiform bacilli in the submucosa of the appendix shown in Fig. 16. ×1200.

Fig. 18.—Hemorrhage, necrosis and leukocytic infiltration in appendix in rabbit, twenty-four hours after intravenous injection of a mixed culture of fusiform bacilli and streptococci from the edematous mesentery in human appendicitis. × 55.

Fig. 19.—Streptococci and fusiform bacilli in the appendix shown in Fig. 18. × 1200.

PLATE 16

Fig. 20.—Colon bacilli in peritoneal coat in a case of subacute appendicitis in man. The organisms resembling diplococci are in partial focus. × 1200.

Fig. 21.—Hemorrhages, necrosis, and leukocytic infiltration in the appendix of rabbit, twenty-four hours after intravenous injection of the colon bacillus isolated from the appendix in a case of colon bacillus appendicitis in man (Case 203). × 58.

Fig. 22.—Colon bacilli in peritoneal coat of appendix shown in Fig. 21. The organisms resembling diplococci are bacilli in partial focus.

PLATE 12

Fig. 1

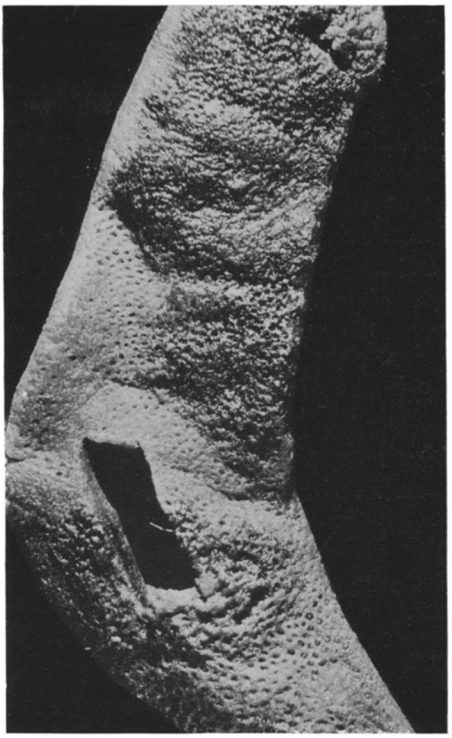

Fig. 2

Fig. 3

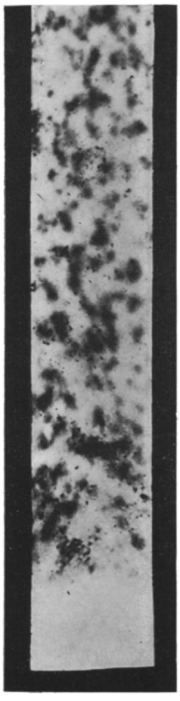

Fig. 4

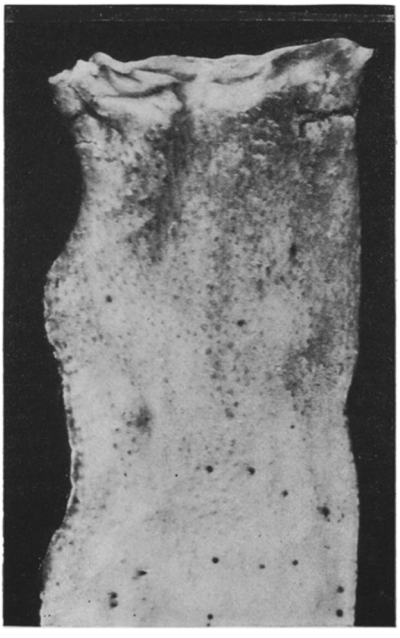

Fig. 5

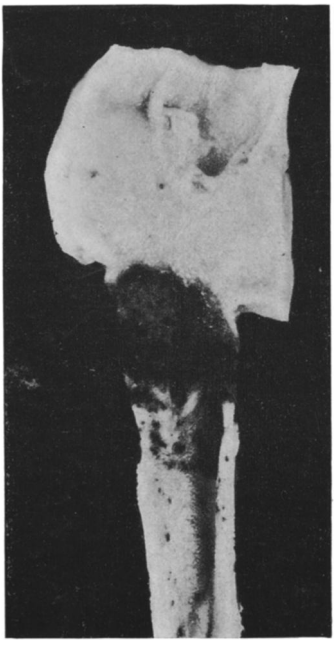

Fig. 6

Fig. 7

Fig. 8

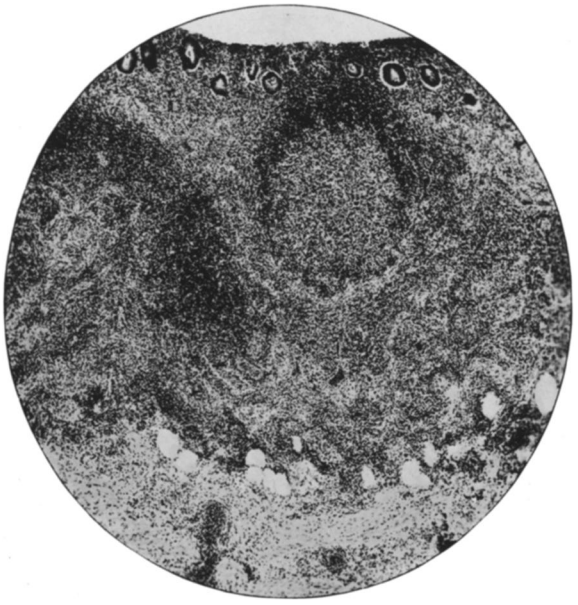

Fig. 9

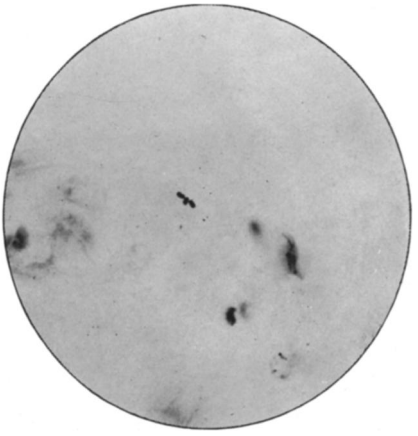

Fig. 10

PLATE 14

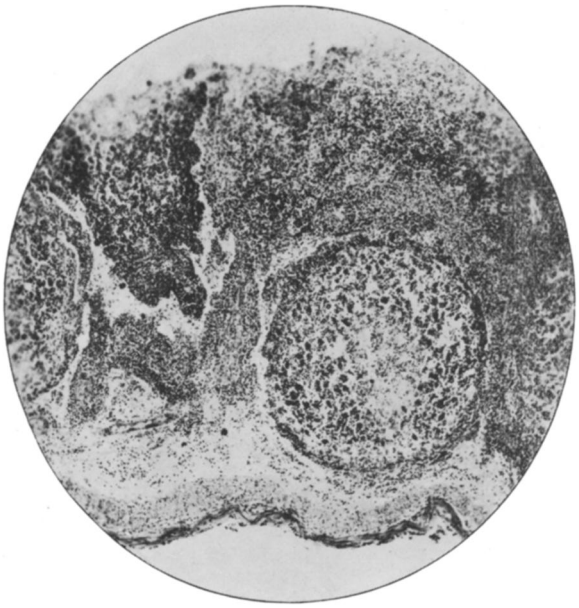

Fig. 11

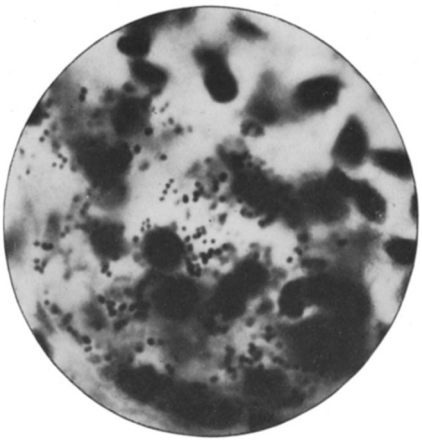

Fig. 12

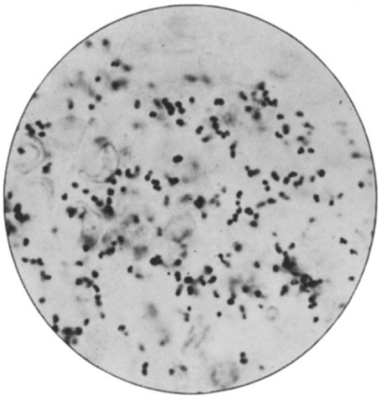

Fig. 13

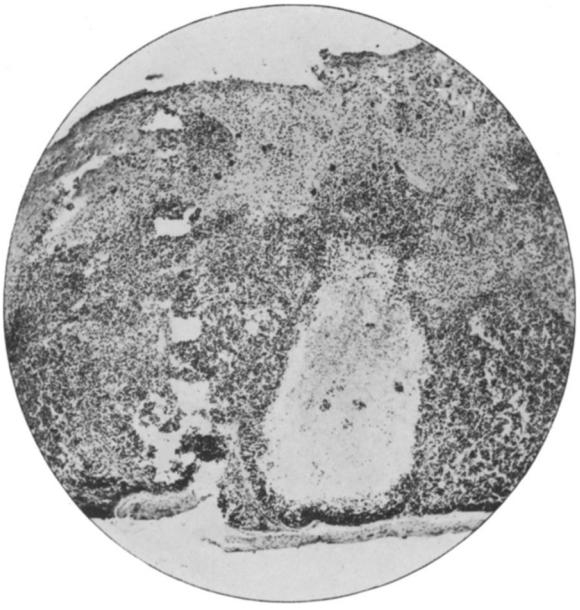

Fig. 14

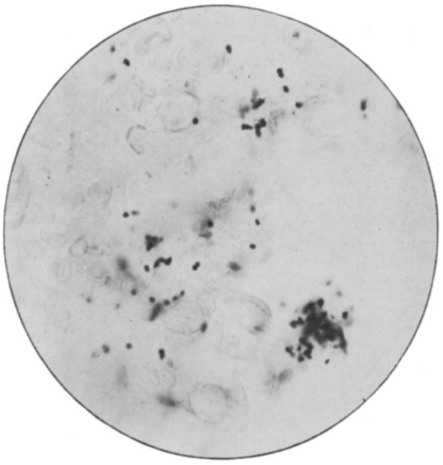

Fig. 15

Fig. 16

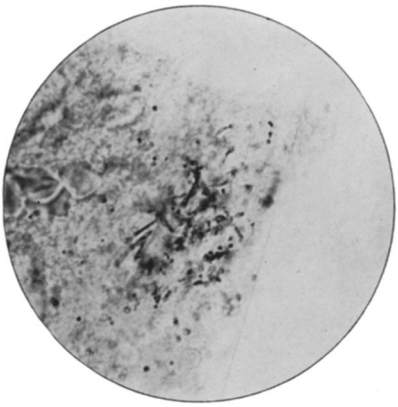

Fig. 17

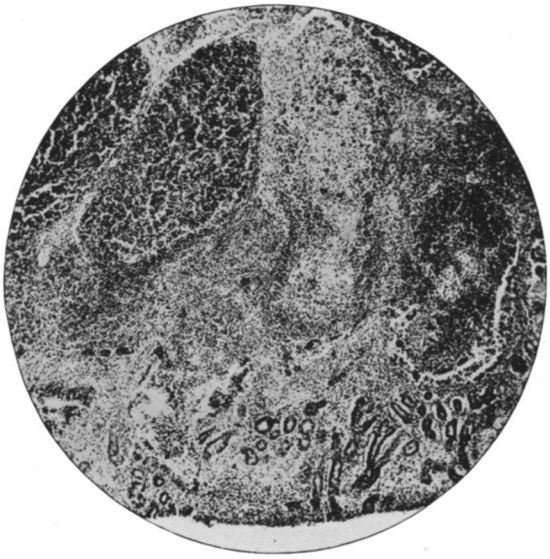

Fig. 18

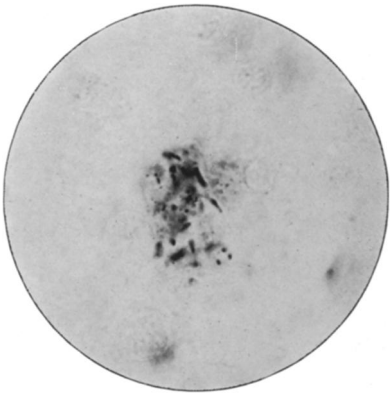

Fig. 19

PLATE 16

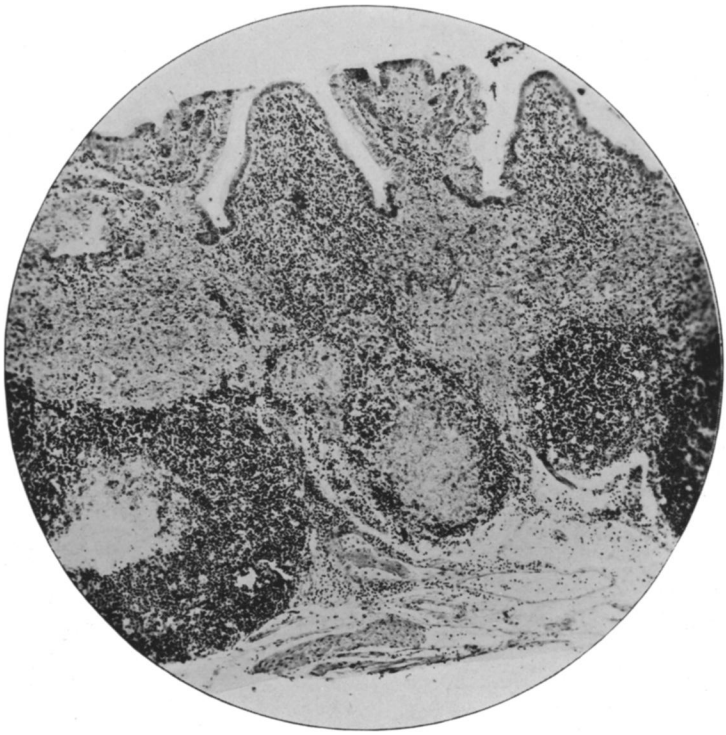

Fig. 21

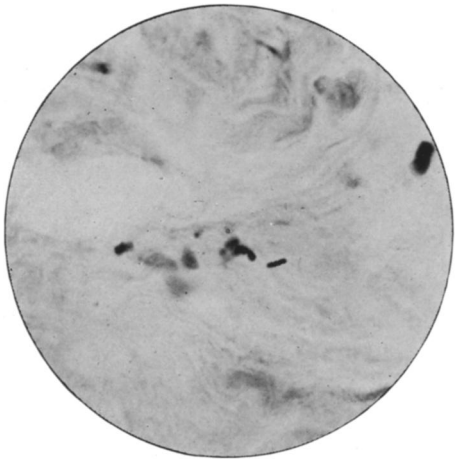

Fig. 20

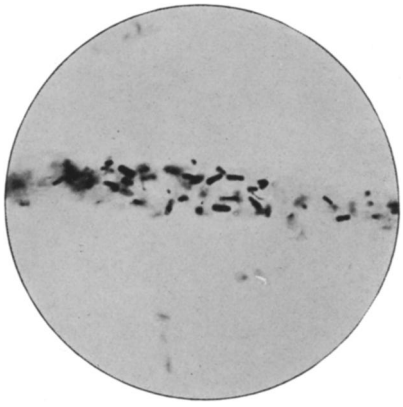

Fig. 22